

## BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDIES OF HYDRA. II. PACEMAKER ACTIVITY OF ISOLATED TENTACLES<sup>1</sup>

NORMAN B. RUSHFORTH AND DONALD S. BURKE<sup>2</sup>

*Department of Biology and Department of Biometry, Case Western Reserve University,  
Cleveland, Ohio 44106*

The tentacles of a coelenterate provide sensory surfaces for the transduction of stimuli from the animal's environment. Their primary role in the capture of prey is well known. Recent work with sea anemones, moreover, emphasizes their importance in complex behavior sequences such as swimming, and their responses to host organisms in commensal relationships (Hoyle, 1960; Robson, 1961a, 1961b; Josephson and March, 1966; Ross and Sutton, 1961).

In the literature at the turn of the century there were sporadic reports that isolated tentacles responded to mechanical and chemical stimuli much in the same way as they did when attached to the animal (Parker, 1896; Üxkull, 1909). Such observations were made long before the techniques of electrical recording from coelenterates became available. However, studies of the behavior of excised actinian tentacles prompted Parker to suggest that "each tentacle contained a neuromuscular mechanism sufficient for its own activity and it is therefore not dependent upon the nervous control of other parts of the animal's body for the production of those movements that it ordinarily exhibits" (Parker 1917, page 95).

Passano and McCullough (1962, 1964, 1965) were the first to record spontaneous electrical activity in *Hydra*. On the basis of such recordings they postulated several pacemaker systems whose interactions control the behavior of the animal. One set of potentials, associated with tentacle contraction, was recorded as small pulses from external electrodes placed on the body column. These Tentacle Contraction Pulses (TCP's) initiated from pacemakers located in the proximal part of each tentacle were observed to sometimes precede a burst of potentials from the Contraction Burst Pacemaker System. This system produced potentials associated with contraction of the hydra column (Passano and McCullough, 1963). The interactions among these two pacemaker systems are discussed in a subsequent paper (Rushforth, in preparation).

The present investigations stemmed from earlier observations that tentacles cut from hydra contracted spontaneously and in response to external stimuli (Rushforth, 1967b). This paper, the second in a series of reports on the behavior and electrical activity of *Hydra* describes patterns of spontaneous potentials in isolated tentacles and modifications of the patterns by external stimuli.

### MATERIALS AND METHODS

The preparations used in these studies were isolated tentacles primarily from two species; *H. pirardi* and *H. pseudoligactis*, (possibly *H. canadensis*). The

<sup>1</sup> Supported in part by grants MH-10734 and GM-12302 from National Institutes of Health.

<sup>2</sup> NSF Undergraduate Research Program Participant, Case Western Reserve University. Present address, Harvard Medical School, Boston, Massachusetts.

methods employed for culturing the hydra were previously described (Rushforth, 1971).

External electrical recordings were made from isolated tentacles using a glass suction electrode (Josephson, 1967). The electrode configuration and methods of recording have been discussed previously (Rushforth, 1971). Efforts to record

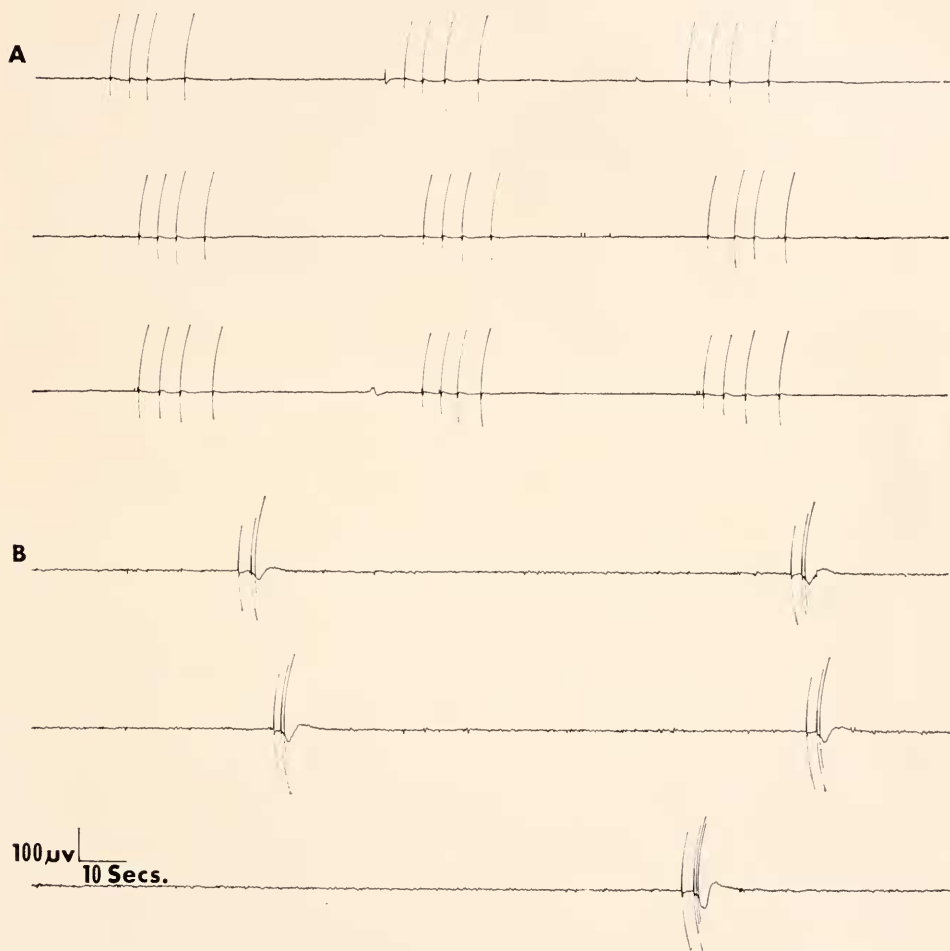


FIGURE 1. Spontaneous electrical activity recorded from isolated tentacles: bursts of TCP's from a *H. pirardi* (A) and a *H. pseudoligactis* (B).

from two sites on a single tentacle were unsuccessful, even when the electrodes used were flexible plastic tubing. Invariably one end of the tentacle would pull out of one of the electrodes during contraction. Thus, it was not possible to measure the conduction velocity of stimulated TCP's.

Overt behavior of tentacles was observed with a dissecting microscope and recorded manually using telegraph keys attached to two channels of a penwriter.

Such observations were made at the same time as recordings of the electrical activity in order to make correlations between behavioral and electrical events. Statistical analyses were performed on the inter-TCP interval measurements taken from recordings of several hours duration using methods described previously (Rushforth, 1971).

A tentacle preparation was sometimes stimulated while its electrical activity and behavior were observed. Photic stimulation was administered using methods previously described (Rushforth, 1971). Specimens of *Artemia*, one at a time, were introduced into the medium above the tentacle to test the effect of live *Artemia salina* nauplii on the spontaneous TCP activity. If an *Artemia* struck the tentacle it sometimes became ensnared by nematocysts and was thus captured. If the *Artemia* placement was made carefully, little mechanical disturbance was introduced to the preparation other than that due to the *Artemia* itself. Reduced glutathione of known concentrations were pipetted into the culture medium to determine the effect of this tripeptide on tentacle behavior and TCP frequency. The preparation was mechanically stimulated by agitating the water in the recording dish with a magnetic stirrer. Turning the stirrer on for brief periods gave pulses of mechanical agitation. Such pulses are observed as discrete increases in the noise level in the electrical record, but do not mask the TCP's emitted by the preparation.

## RESULTS

### *Temporal patterns of spontaneously produced tentacle contraction pulses*

(1) *Inter-pulse interval distributions.* The electrical activity of the isolated tentacles of both species consisted exclusively of large compound pulses, up to 10 mv in amplitude and of approximately 200 msec duration. Visual observations of the preparation indicated that the pulses were associated in a one-to-one fashion with symmetric contractions of the tentacle. These potentials therefore are TCP's, recorded directly from tentacles. They are much larger than TCP's observed in records taken with external electrodes placed on the base of the hydra body column. Records taken from the base of the isolated tentacle consisted primarily of bursts of TCP's. No potentials similar to RP's were observed. The typical burst patterns of TCP's are illustrated in Figure 1 for an isolated tentacle of *H. pirardi* (A), and *H. pseudoligactis* (B). The patterns of electrical activity taken from isolated tentacles are more homogeneous both within a single preparation and among the various tentacles of the same species, than those of the Column Contraction System (see Rushforth, 1971). However, there is variability in the numbers of pulses per burst, the interburst interval lengths and among the interval lengths between successive pulses in a burst.

Measurements of the interval lengths (to the nearest 0.4 second) between successive TCP's were made from records of two or three hours duration for isolated tentacles of *H. pirardi* and from records of approximately 8 hours duration for *H. pseudoligactis*. Plots of inter-pulse histograms and first order joint interval scatter diagrams were constructed for each preparation in the sample (*H. pirardi*  $n=7$ , *H. pseudoligactis*  $n=5$ ). Figures 2 and 3 represent such plots for TCP's from a single isolated tentacle of *H. pirardi* and one from *H. pseudoligactis*. The

histograms depicting the percentage of inter-TCP intervals in ten second classes constitute the lower graphs in the two figures. Both histograms are bimodal. Bimodality of the inter-pulse interval histograms and L shaped first order joint interval scatter diagrams (upper graphs) are characteristic of a pattern made up of bursts of pulses (Rushforth, 1971).

For both species the initial modal class of the lower graph includes intervals less than ten seconds in length and is, therefore, made up of intervals between

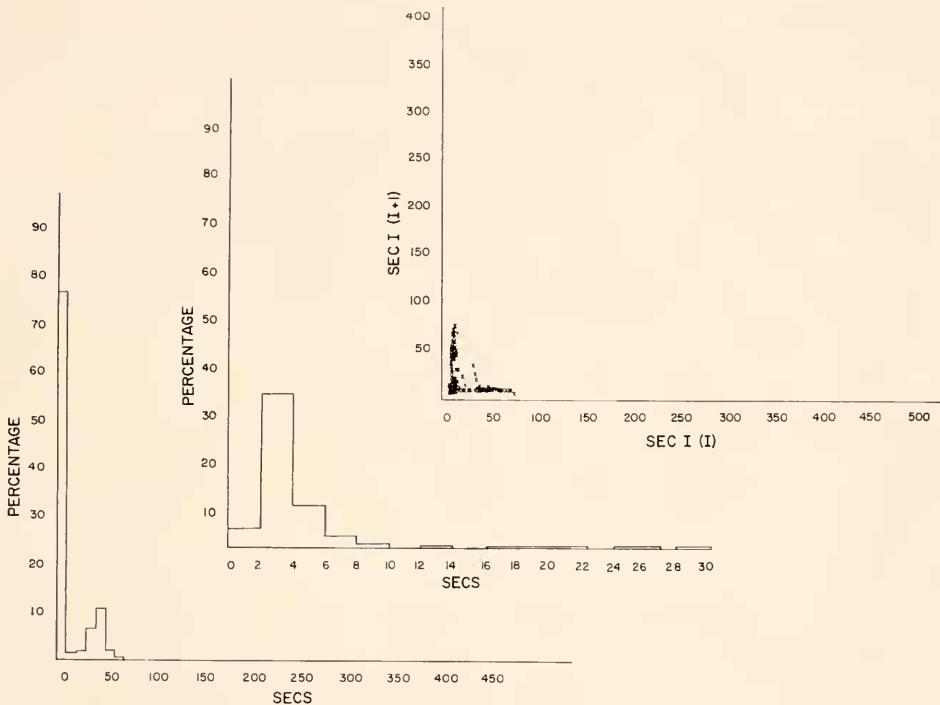


FIGURE 2. Inter-pulse interval distributions for TCP's from an isolated tentacle of *H. pirardi*. The lower histogram is a plot of the percentage of intervals falling in 10 second interval classes for all intervals in a four hour record. The middle histogram consists of intervals less than 30 seconds in length from this record. It is a plot of the percentage of intervals falling in two second classes. The upper graph is the first order joint interval scatter diagram for all intervals in the record.

pulses of a burst. These intervals comprise 76 and 72 per cent of all the intervals for the two respective records. A secondary modal class of 40-50 seconds for *H. pirardi* contains about 10 per cent of the intervals. These intervals are the inter-burst intervals. In the *H. pseudoligactis* preparation a relatively smaller secondary modal class occurs between 20 and 30 seconds, making up only 3-4 per cent of all interval lengths. A much larger percentage (65 per cent) of inter-pulse intervals longer than 10 seconds have lengths greater than a minute. The middle histograms depict the percentage distributions of those inter-TCP intervals that are less than 30 seconds. These two histograms show a marked

difference in interval patterns for the two species. The distribution of interval lengths for *H. pirardi* is unimodal (Modal class 3–4 seconds) and roughly symmetrical, while in *H. pseudoligactis* most of the intervals are less than one second in length and the distribution is skewed to the right.

In both species the great majority of short intervals, those that occur within bursts, are less than 10 seconds. Ten seconds was therefore chosen as the cut-off point to classify intervals as either long or short intervals. A burst was defined as two or more pulses with successive inter-pulse interval lengths less than 10 seconds.

(2) *Inter-pulse interval measurements.* Calculations were made of various inter-pulse interval measurements for the samples of both species. These measure-

TABLE I  
*Measurements of intervals between tentacle contraction pulses for isolated tentacles of H. pirardi and H. pseudoligactis (The long interval lengths and inter-burst interval lengths differ because occasionally two or more long intervals occurred between bursts)*

Measurement	Mean (Standard error)	
	<i>H. pirardi</i> (n = 7)	<i>H. pseudoligactis</i> (n = 5)
Number of intervals/12 hr	3565 (806)	1075 (200) $P < 0.001$
Short interval lengths (sec)	3.6 (0.3)	2.0 (0.4) $P < 0.05$
Long interval lengths (sec)	41.5 (5.8)	194.1 (31.6) $P < 0.01$
Per cent of intervals classified as long intervals	25.4 (0.7)	25.9 (1.5) $P > 0.10$
Number of intervals per burst	3.1 (0.1)	3.7 (0.2) $P < 0.05$
Mean interval length per burst (sec)	3.7 (0.1)	1.9 (0.4) $P < 0.01$
Burst length (sec)	11.4 (0.9)	7.4 (1.9) $P > 0.05$
Inter-burst interval length (sec)	42.2 (5.6)	208.5 (26.1) $P < 0.001$

ments are based on approximately two hour records from *H. pirardi* tentacles, and on approximately 8–12 hour recordings for the tentacles of *H. pseudoligactis*. The recording times were longer with *H. pseudoligactis* because of the lower frequency of TCP in this species. Mean values for the interval parameters together with their standard errors are given in Table I.

Several marked differences between the patterns of spontaneously produced TCP's in the two species are evident from Table I. The TCP frequencies, as measured by the numbers of TCP intervals/12 hours, are significantly greater ( $P < 0.001$ ) for *H. pirardi* than for *H. pseudoligactis*. The interval lengths of short intervals (those less than 10 seconds in duration) are significantly ( $P < 0.01$ ) longer for *H. pirardi*. This gives rise to a significantly longer mean interval length for the intervals within bursts ( $P < 0.01$ ) for this species. However, there are more intervals within a burst for *H. pseudoligactis* ( $P < 0.05$ ). The net effect of these two factors results in approximately the same burst lengths for the two species. The lengths of the long intervals (those greater than 10 seconds)

are much greater in *H. pseudoligactis*, reflected in a significantly greater mean inter-burst interval ( $P < 0.01$ ). However, the percentages of all intervals which are greater than 10 seconds in length are very similar for all tentacles in both species.

(3) *Lengths of short intervals in a TCP burst.* With CP bursts recorded from the column the inter-pulse intervals first become smaller and then lengthen again toward the end of the burst (Passano and McCullough, 1963, Rushforth, 1971). The intervals within TCP bursts from isolated tentacles behave somewhat similarly.

In Table II, the mean values of interval lengths for bursts having differing numbers of intervals are presented as a function of the order of the interval within

TABLE II  
*Relationship between interval length and position of the interval in a burst for isolated tentacles of H. pirardi and H. pseudoligactis*

Number of short intervals per burst	Mean interval (sec) Position of interval in the burst						Number of bursts
	1	2	3	4	5	6	
A <i>H. pirardi</i>							
1	5.3						45
2	3.5	3.7					273
3	3.0	2.6	4.4				1049
4	2.8	2.3	3.5	5.5			346
5	2.7	2.4	3.2	4.2	4.5		48
B <i>H. pseudoligactis</i>							
1	2.4						119
2	2.5	1.2					188
3	1.2	1.1	1.8				274
4	1.1	0.7	1.2	4.0			160
5	1.2	0.9	1.3	3.1	4.4		84
6	2.2	1.0	1.0	2.5	2.9	4.0	35

a burst. For *H. pirardi*, the mean values are obtained by pooling the bursts for a sample of seven preparations of this species. For *H. pseudoligactis*, mean values are based on bursts pooled from five preparations. Based on an analysis of variance test, mean interval lengths are significantly different ( $P < 0.01$ ) among the various intervals in the bursts. This result applies in all cases except for bursts consisting of two intervals in *H. pirardi* isolated tentacles. In general, the second interval in a burst is shorter than the first interval, while succeeding intervals systematically increase in length. In every case, except bursts of two intervals for *H. pseudoligactis*, the last interval is longer than the other intervals in the burst. This consistent pattern is observed in spite of the previously described inter-species differences in the activities of the Tentacle Contraction Pacemaker System.

(4) *Temporal pattern of contraction pulses from segments of a tentacle.* The entire structure of the excised tentacle is not required for the production of TCP's. Bursts of potentials may be recorded from segments cut from a tentacle. The patterns of TCP firing from such preparations are quite similar to those of



the entire isolated tentacle. For example, a distal segment, approximately  $\frac{1}{5}$  the length of the whole *H. pseudoligactis* tentacle, gave histograms and a first order joint scatter diagram very similar to those of an intact isolated tentacle of this species (Fig. 4). The inter-pulse histograms are bimodal and the first order joint interval scatter diagram gives the L-shaped plot characteristic of bursts of potentials. Thus, TCP distributions from segments of a tentacle are similar to those of the entire tentacle.

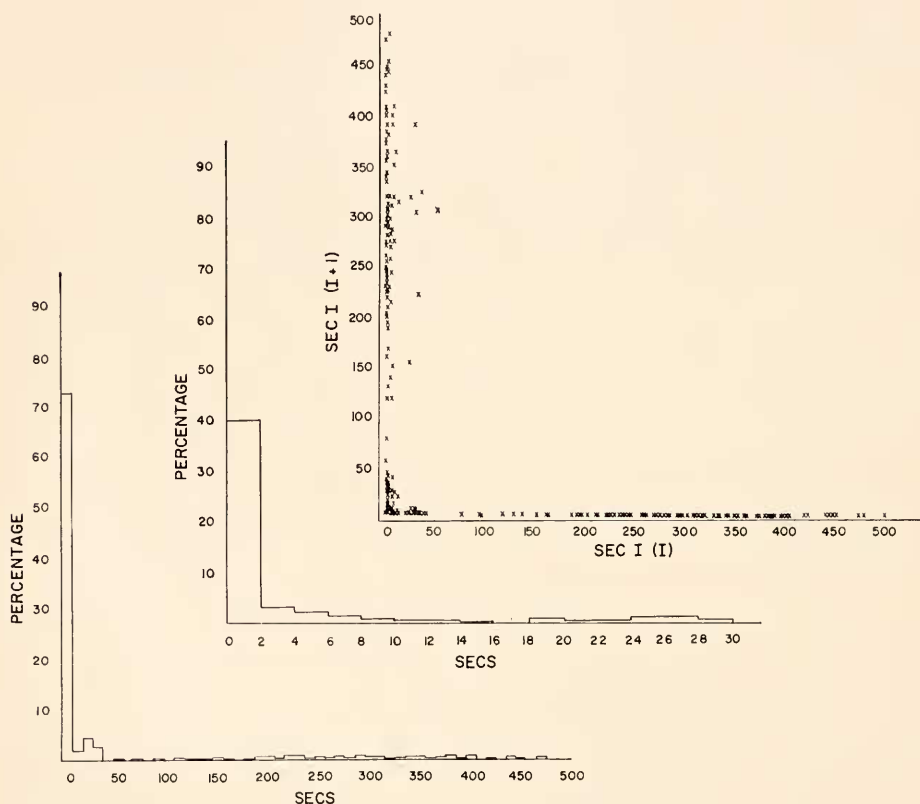


FIGURE 3. An inter-pulse interval representation like that of Figure 2 for *H. pseudoligactis*. The data is from a 12 hour continuous recording.

### *Changes of TCP activity with external stimulation*

(1) *Effects of light stimulation.* The TCP activity patterns are changed when isolated tentacles of *H. pirardi* and *H. pseudoligactis* are exposed to a regime of two minute periods of direct illumination interspersed with two minute periods of ambient light. In both species, TCP's are more frequent during the strong light periods. This is consistent with the finding that direct illumination increases the TCP frequency from attached tentacles in the two species, (Rushforth, in preparation). Examples of the electrical records of isolated tentacles exposed to such light

stimulation are given in Figure 5, (A. *H. pirardi*; B. *H. pseudoligactis*). It is seen that for three sequences, consisting of two minutes of direct light followed by two minutes of ambient light, TCP pulses are more numerous in the periods of direct illumination.

Features of TCP activity in both periods of direct and ambient illumination are summarized in Table III. The pulse frequency, burst frequency, and number of intervals per burst are all significantly greater ( $P < 0.01$ ) in the periods of direct illumination. However, there is no significant difference ( $P > 0.10$ ) in

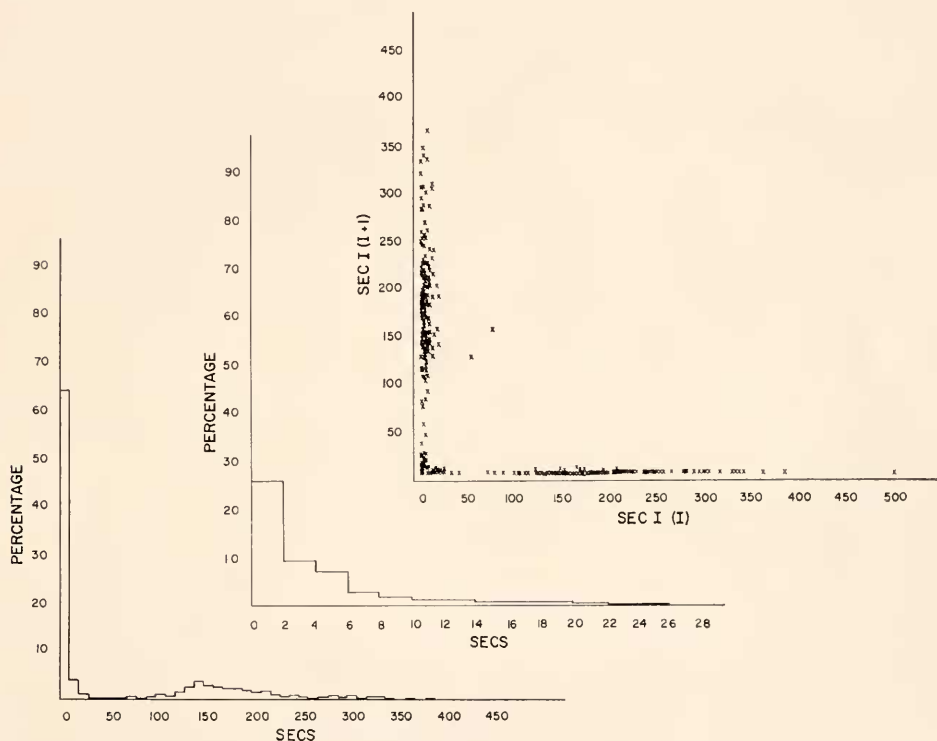


FIGURE 4. An inter-pulse interval representation like that of Figure 2 for a small distal portion of a *H. pseudoligactis* tentacle. The data is from a 12 hour recording.

the mean interval/burst. The same systematic relationship between the interval length and its position in the burst, noted previously for spontaneously produced bursts of TCP's, was observed for light stimulated tentacles. For both species, intervals are shorter in mid-burst than at the start or end of the burst.

In order to verify that direct illumination of an isolated tentacle increases the number of pulses within a burst, preparations were exposed to strong light at different points in the burst-inter-burst cycles. Preparations of *H. pirardi* were illuminated directly following the first pulse in a spontaneously produced burst.

At other times the preparations were exposed to strong light at random points in time and the light was turned off following the first pulse in the light-induced



burst. The numbers of pulses/burst and the mean interval/burst for bursts stimulated by these two methods, are compared with "control" bursts. The groups of control bursts were composed of bursts immediately preceding and immediately following both types of light modulated bursts. Comparisons were based on 20-50 bursts for both types of stimulation for five individual tentacles. It was found that the number of pulses per burst was significantly increased ( $P < 0.01$ ) when the preparation was exposed to strong light after the first pulse in a spontaneous burst (mean = 5.4, S.E. = 0.1). Conversely, the number of pulses per burst is significantly decreased ( $P < 0.01$ ) when light is removed after the first pulse in a light induced burst (mean = 2.9, S.E. = 0.1). These values were compared to the average values of control bursts immediately preceding and following light stimulation (mean = 3.9, S.E. = 0.1). The two modes of light stimulation do not significantly ( $P > 0.10$ ) affect the mean interval length/burst.

TABLE III

*Effects of light on TCP bursts in isolated tentacles of H. pirardi and H. pseudoligactis*

	Mean (S.E.) based on five preparations for each species		
	Direct illumination	Ambient illumination	
a. <i>H. pirardi</i>			
Number of TCP's/2 min	13.6 (0.6)	9.0 (0.6)	$P < 0.01$
Number of bursts/2 min	2.9 (0.1)	2.2 (0.1)	$P < 0.01$
Number of intervals/burst	3.7 (0.1)	3.1 (0.1)	$P < 0.01$
Mean interval/burst	3.5 (0.1)	3.5 (0.1)	$P > 0.10$
b. <i>H. pseudoligactis</i>			
Number of TCP's/2 min	11.3 (0.6)	6.2 (0.9)	$P < 0.01$
Number of bursts/2 min	2.3 (0.1)	1.4 (0.2)	$P < 0.01$
Number of intervals/burst	3.9 (0.1)	3.4 (0.1)	$P < 0.05$
Mean interval/burst	2.0 (0.1)	1.8 (0.1)	$P > 0.10$

In addition, in both cases intervals are shorter in mid-burst than at the start or end of the burst.

(2) *Effects of mechanical stimulation.* Single TCP's can be induced with weak mechanical stimuli to isolated tentacles and bursts of TCP's can be initiated with stimuli of greater intensity (Fig. 6). At high stimulus frequencies (greater than 1/15 sec) the response to mechanical stimulation rapidly adapts. Induction of the TCP system to fire as single pulses, using mechanical stimulation, is associated with inhibition of TCP bursts. However, with cessation of mechanical stimulation, the normal spontaneous burst pattern of TCP production is soon restored (Fig. 6).

(3) *Effects of feeding stimuli.* Specimens of *Hydra* undergo complex feeding reactions following the capture of a prey organism by nematocyst discharge from the tentacles. The following experiments were undertaken to determine the effects on the behavior and electrical activity of isolated tentacles of stimuli which elicit feeding reactions in hydra.

Initial behavioral observations made in the absence of electrical recordings indicated that placement of a single *Artemia salina* nauplius on an isolated tentacle of *H. pseudoligactis* inhibited the normal spontaneous contraction burst activity.

While bursts of longitudinal contractions were suppressed, the tentacle underwent a considerable number of asymmetric contractions. These contractions were very similar to the writhing activity frequently observed in the tentacles of an intact hydra during the middle stages of the feeding response. Invariably after several

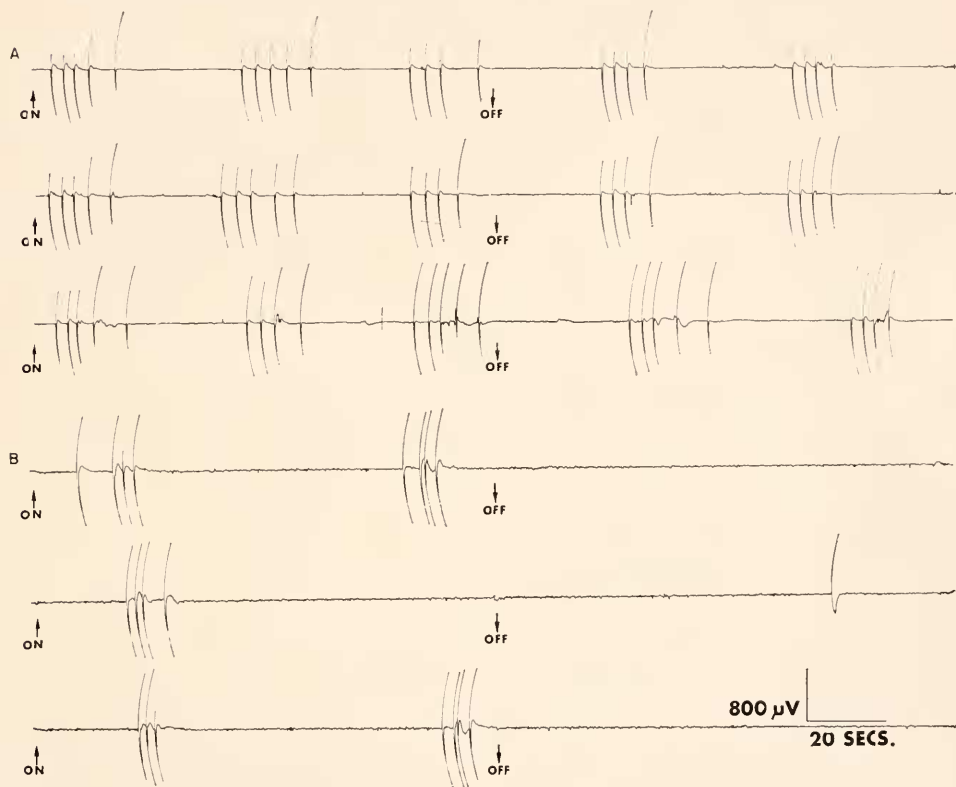


FIGURE 5. Effects of light stimulation on TCP firing. Frequency of bursts of TCP's in two minute periods of direct illumination (on to off) compared with the frequency in ambient light, for isolated tentacles *H. pirardi* (A, upper three records) and *H. pseudoligactis* (B, lower three records).

minutes of writhing activity the specimen of *Artemia* would detach from the tentacle and fall inertly to the bottom of the dish. The tentacle's contractile behavior gradually changed from uncoordinated contractions to the normal contraction burst activity. The behavioral sequence just described may be elicited equally well with aqueous extracts of *Artemia salina* or by administering reduced glutathione in concentrations of  $10^{-5}$  M GSH to the isolated tentacles.

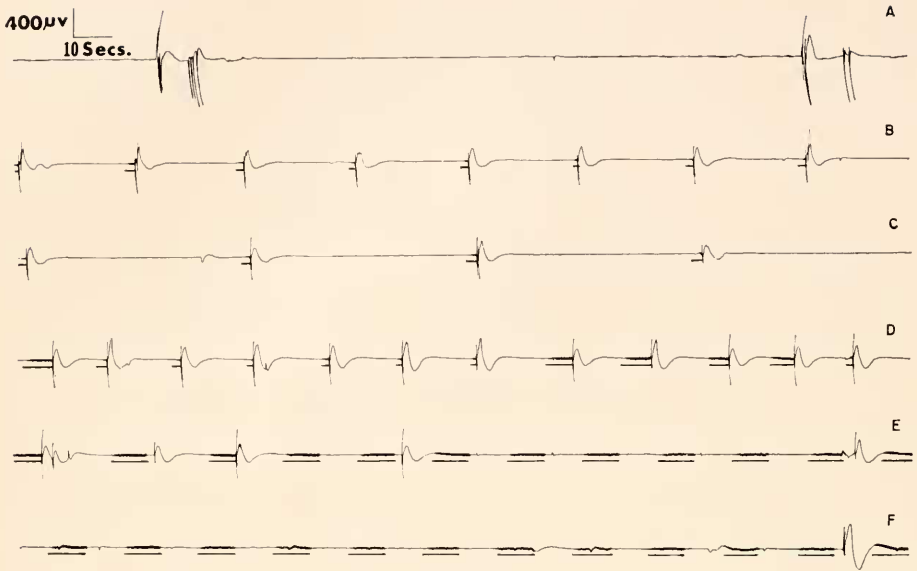


FIGURE 6. Effects of mechanical stimulation on TCP firing in an isolated tentacle of *H. pseudoligactis*. Spontaneous bursts of TCP's (A) and single TCP's induced by agitation of the surrounding culture medium (B, C, D, E, and F). The increase in the noise level and the unbroken lines below each electrical record show the duration of stimulation. Adaptation of the preparation to mechanical stimulation occurs in E. and F.

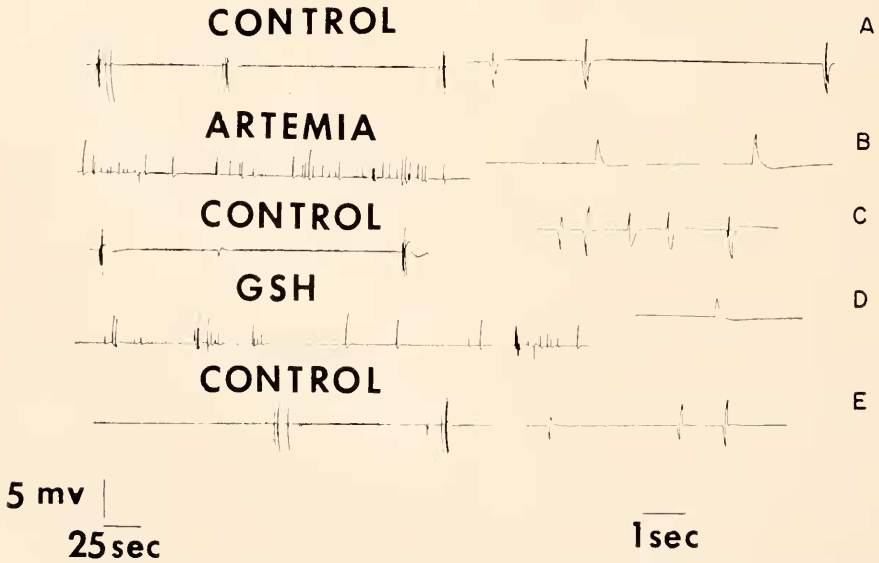


FIGURE 7. Effects of *Artemia salina* nauplii and  $10^{-5}M$  GSH on the electrical activity of an isolated *H. pseudoligactis* tentacle. Records on the right hand side of the figure were

The records in Figure 7 show the effects of an *Artemia salina* nauplius and  $10^{-5}$  M GSH on the electrical activity of an isolated *H. pseudoligactis* tentacle. The short sequences in the right hand portions of each record were taken at a higher recording speed to show the shapes of the pulses. Monophasic pulses associated with tentacle writhing supplant the normal pattern of spontaneously produced multiphasic TCP's. During the initial phases of the response these pulses are relatively frequent (every 1–3 sec). They occur primarily as single events, and have lower amplitude than TCP's, usually no greater than one millivolt. Gradually, however, TCP's return to the record, sometimes first as single pulses and then later as bursts. The monophasic pulses decrease in frequency and the burst pattern is completely restored. This follows release of the nauplius from the tentacle, although no abrupt change in the electrical activity of the preparation is correlated with the event of prey release. In the case of  $10^{-5}$  M GSH the TCP burst pattern usually returns within 15–20 minutes after addition of GSH.

Inhibition of TCP bursts on capture of a single *Artemia* is reflected by significantly lengthened ( $P < 0.01$ ) inter-burst interval. The inter-burst interval length is 3–5 fold greater than pre-stimulation values. After prey release, however, the subsequent ten inter-burst intervals are significantly smaller ( $P < 0.01$ ) than those of corresponding inter-burst intervals preceding stimulation, suggesting post inhibitory excitation of the TCP pacemaker system in producing bursts.

#### DISCUSSION

(1) *Spontaneously produced TCP's.* Isolated tentacles produce bursts of potentials, while tentacles attached to the hydra produce both single pulses and bursts (Rushforth, in preparation). Surgical removal of the tentacles from the animal presumably excites the TCP system, and placing the wounded preparation in the suction electrode puts it in a stressful recording situation. The CP system of *H. pseudoligactis*, which normally produces single pulses, is induced to give bursts of potentials when exposed to increasing basal pressure in a suction electrode (Rushforth, 1971). Thus, the initial TCP burst pattern may reflect prior wounding and electrode suction. However, sequences of bursts have been recorded continuously from a tentacle for more than 24 hours after removal from the animal, long after wound healing and adaptation to the electrode should have taken place. Thus, the TCP burst pattern may reflect the removal of the electrical input from other pacemakers, rather than excitation from the initial wounding of the preparation.

In both species investigated the pattern of TCP's from isolated tentacles consisted of bursts of potentials. As with the Column Contraction System, however, the temporal characteristics of TCP's are quite different in *H. piriardi* and *H. pseudoligactis*. TCP frequencies are much greater for *H. piriardi* as are the interval lengths within a burst. However, this species has fewer pulses per

---

taken at a higher recording speed to show the shapes of the pulses; A. spontaneous bursts of TCP's; B. monophasic pulses observed when the tentacle writhes after capturing an *Artemia*; C. restoration of the normal burst pattern of TCP's after the *artemia* has been released and writhing has terminated (30 minutes after B was taken); D. monophasic pulses induced by  $10^{-5}$  M GSH (5 minutes after C was recorded); E. normal TCP burst pattern 30 minutes after the addition of GSH.

burst and significantly shorter inter-burst intervals than *H. pseudoligactis*. If the mean interval per burst and the inter-burst interval are plotted, points derived for the TCP patterns of the two species, form two non-overlapping sets (Fig. 8).

The entire structure of the isolated tentacle is not necessary for the production of the characteristic burst pattern of TCP's. Small blocks of tissue from the tentacles give patterns representative of the species. This suggests that potential pacemakers occur in different parts of a tentacle and that they all have similar properties.

(2) *Modifications in TCP burst patterns with external stimuli.* The effect of strong light stimulation increases the frequency of TCP's in isolated tentacles

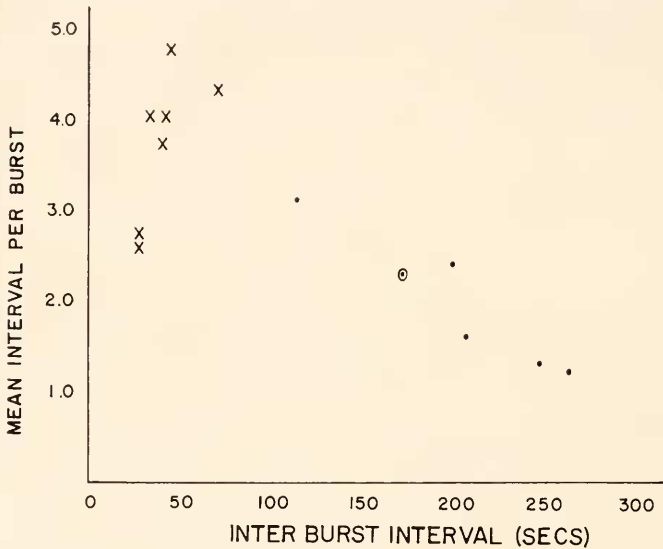


FIGURE 8. Mean interval per burst and inter-burst interval length for isolated tentacles of *H. pirardi* (crosses) and *H. pseudoligactis* (closed circles). The large closed circle represents values for a distal segment of tissue cut from an isolated tentacle of *H. pseudoligactis*.

of both species. Both the frequency of bursts and the number of intervals per burst are increased by such stimulation. However, the mean interval length is unchanged as is the systematic relationship between the interval length and its position in the burst-inter-burst cycle, found in spontaneously produced bursts of TCP's. Light stimulation at different points in the burst-inter-burst cycle may be used to add additional pulses to bursts or subtract pulses from the normal burst size. Thus, light can effect both mechanisms determining the occurrence of bursts and those controlling the production of pulses within the bursts.

Previous studies have shown that the tentacles are controlling factors in the response of the intact hydra to mechanical stimulation (Rushforth, 1965). When all the tentacles are removed the animal does not contract to such stimulation, suggesting that the initiation of mechanically induced contractions resides in the

tentacle structure. In the present study it has been shown that isolated tentacles respond to mechanical agitation, changing their pattern of bursts of potentials to one of single pulses synchronized with the mechanical stimuli. As in the case of the whole animal this preparation adapts to repeated stimulation. With such adaptation the normal pattern of bursts of TCP's is restored. Adaptation of tentacles to mechanical stimulation and the associated changes in electrical activity may underlie the processes of habituation of the whole animal found on repeated stimulation (Rushforth, 1967a).

Inhibition of TCP bursts in isolated tentacles is also found with feeding stimuli. Asymmetric contraction potentials are elicited by live specimens of *Artemia*, aqueous extracts of *Artemia*, or the tripeptide reduced glutathione. The effects of these stimuli in producing feeding responses has been extensively studied by Lenhoff (1961, 1968). The present studies show an effect of the stimuli on an isolated pacemaker system in the animal.

The mechanisms underlying the production of the pulses associated with asymmetric movements comprising the uncoordinated writhing activity of isolated tentacles are unknown. While occasionally isolated pulses will be observed in the record of the unstimulated tentacle, their appearance on exposure to feeding stimuli is quite dramatic. There is insufficient evidence to indicate that they are produced by a discrete pacemaker system separate from the TCP System. It is possible that the pulses result from chemostimulatory effects on the TCP System, causing alterations in firing loci and the spread of conduction to neighboring pacemaker units. The net effect may be to partially block the normal mode of firing of the pacemaker system. Indirect evidence for this idea comes from the fact that their appearance is always associated with inhibition of bursts of TCP's. In absence of direct evidence, however, this remains purely a conjecture.

On first observation it was thought that the monophasic pulses might be associated with the coordinated "concert" movements of the hydra tentacles, which are characteristic of the feeding response. Concert activity has been found to be increased by feeding stimuli (Rushforth and Hofman, 1966). The monophasic pulses which were recorded were accompanied by writhing rather than smooth orally directed sweeping concert movements. Passano and McCullough (1963) observed that tentacle writhing was frequently a component of the light response of intact hydras. Such behavior was not accompanied by concerts, thus emphasizing the separateness of writhing and concert activity. Experiments with isolated tentacles show that after inhibition of TCP bursts by feeding stimuli there is increased excitability of the TCP burst system. This post inhibitory excitability has been observed with feeding stimuli in the case of the CP System (Rushforth, in preparation) and is reminiscent of processes of post inhibitory rebound seen in neural systems (Erlanger and Glasser, 1937).

(3) *Models of pacemaker discharge.* Several types of central neurons fire in clusters of spikes, separated by long intervals. Such bursts of potentials have been described in such diverse preparations as: mammalian hippocampal cells (Kandel and Spencer, 1961); locust flight motoneurons (Wilson, 1964); lobster cardiac ganglion neurons (Maynard, 1955); interneurons in the abdominal cord of the crayfish (Kennedy and Takeda, 1965); crustacean motoneuron preparations (Ikeda and Wiersma, 1964; Gillory and Kennedy, 1969, Davis, 1969); and the



"parabolic burster" cell of *Aplysia* (Strumwasser, 1965). Frequently the pattern of such bursts has been shown to be endogenous to the cell and not due to periodic synaptic input. Mechanisms of super-excitability after an impulse (Hodgkin, 1948, Wright and Coleman, 1964), depolarizing after-potentials (Kandel and Spencer, 1961, Kennedy and Mellon, 1964), and periodic changes in the conductivity for specific ions (Strumwasser, 1968) have been suggested for the production of such patterned discharge.

During the past five years considerable interest has arisen from the development of models describing the firing patterns of isolated neurons (Geisler and Goldberg, 1966, Junge and Moore, 1966, Calvin and Stevens, 1967, Stein, 1967). A common feature of such models is the specification that a pulse is generated when some measure of cell excitability, for example the membrane potential, reaches some critical threshold level. In most models the threshold is a time dependent function containing parameters of absolute and relative refractory periods. All of the models have the basic assumption of random elements in the pulse generating processes. Models specifically constructed to describe bursts of potentials have been described by Strumwasser, (1968) and Davis, (1969). Such models for bursts incorporate the basic features of the simpler models for single pulses, but assume additional components. In the case of bursts of potentials from swimmeret motoneurons in the lobster, the model assumes sinusoidal excitation to motoneuron units. This model has been successfully used in computer simulation studies to reproduce the temporal characteristics of observed discharge patterns (Davis and Murphy, 1969).

Quantification of the pulse patterns places some limits on the kinds of models which might be proposed to generate such patterns. A successful model must account for the following features: (1) there must be a mechanism to initiate and to terminate bursts. The termination of bursts may be a result of negative feedback as has been proposed in a model for the CP System (Rushforth, 1971); (2) short term changes in firing parameters must be included to account for variability in the number of pulses per burst, and in the inter-burst interval length. Such parameter changes must be consistent with the modifications in burst-inter-burst characteristics effected by light stimulation, *i.e.*, simultaneous increase in pulse number and reduction in interburst interval length; (3) initial decrease and later increase in pulse intervals within the burst, seen in both unstimulated TCP's and those induced by light, must be simulated by the discharge patterns derived from the model. This relationship is a constant feature of both TCP and CP bursts, and has been observed in other hydroids (Morin, 1969), and in other invertebrate systems (Strumwasser 1965, Davis 1969). This functional relationship was the restriction giving rise to the assumption of sinusoidal input in the model of bursts for swimmeret motoneurons in lobsters (Davis and Murphy, 1969).

Our experiments suggest that while the number of pulses per burst and the inter-burst interval length are quite variable components of TCP discharge patterns, the mean interval per burst and the ordered lengths within a burst are constant properties of the output. Neither can easily be experimentally changed even using patterned stimulation.

The results of these studies strongly demonstrate the similarity of the TCP System in isolated tentacles to that of the CP System of the hydra body column, particularly in the case of *H. pirardi*. Temporal characteristics of spontaneously produced bursts of pulses and their modification with external stimuli, are very similar for the two systems. It seems quite likely that the mechanisms of pulse production for TCP's are the same as those of CP generation in the body column. The similar size of the potentials, their duration and compound shape together with the temporal characteristics all point to similar mechanisms of production. Thus the model originally suggested for the CP System (Rushforth, 1971) might be an appropriate one for the TCP System.

In this paper we have described the temporal characteristics of spontaneously produced electrical potentials from an isolated hydroid pacemaker system and their modification with external stimuli. In future studies, isolated pacemaker systems in other hydroids will be investigated to determine the generality of the features observed here for this preparation. In addition we will investigate the interactions between different pacemaker systems. By such means we hope to gain insight into the coordinating mechanisms controlling behavior in coelenterates, the most simply organized living animals with an indisputable nervous system.

#### SUMMARY

1. Electrical potentials associated with coordinated contraction of the longitudinal muscles can be recorded from isolated tentacles of *Hydra*. In both *H. pirardi* and *H. pseudoligactis* these Tentacle Contraction Pulses (TCP's) occur chiefly in bursts, which are more frequent in *H. pirardi*. The bursts are approximately the same length in the two species but those of *H. pirardi* contain more pulses.

2. Inter-pulse interval lengths within a burst decrease but then lengthen again toward the end of the burst. Small segments of a tentacle produce the same pulse patterns as whole tentacles, indicating that there are a number of potential pacemakers with similar properties dispersed within a tentacle.

3. Strong illumination increases the burst frequency and the number of pulses per burst. Pulses of mechanical stimuli induce single TCP's and inhibit TCP bursts. Isolated tentacles habituate to repeated mechanical stimuli. Exposure of the tentacle to live or aqueous extracts of *Artemia* nauplii, or  $10^{-5} M$  GSH inhibits TCP bursts and monophasic pulses are induced which are associated with asymmetric writhing movements.

#### LITERATURE CITED

- CALVIN, W. H., AND C. F. STEVENS, 1967. Synaptic noise as a source of variability in the interval between action potentials. *Science*, **155**: 842-844.
- DAVIS, W. J., 1969. The neural control of swimmeret beating in the lobster *J. Exp. Biol.*, **50**: 99-117.
- DAVIS, W. J., AND R. K. MURPHY, 1969. Discharge patterns of swimmeret motoneurons in the lobster, simulated with a digital computer. *J. Exp. Biol.*, **50**: 119-128.
- ERLANGER, J., AND H. S. GLASER, 1937. Electrical signs of nervous activity. University of Pennsylvania Press, Philadelphia, 221 pp.
- GEISLER, C. D., AND J. M. GOLDBERG, 1966. A stochastic model of repetitive activity of neurons. *Biophys. J.*, **6**: 53-69.

- GILLORY, H. L., AND D. KENNEDY, 1969. Pattern generation in a crustacean motoneuron. *J. Neurophysiol.*, **32**: 595-606.
- HOYLE, G., 1960. Neuromuscular activity in the swimming sea-anemone, *Stomphia coccinea* (Müller). *J. Exp. Biol.*, **37**: 671-688.
- HODGKIN, A. L., 1948. The local electrical changes associated with repetitive action in a non-modulated axon. *J. Physiol.*, **107**: 165-181.
- IKEDA, K., AND C. A. G. WIERSMA, 1964. Autogenic rhythmicity in the abdominal ganglia of the crayfish: the control of swimmeret movements. *Comp. Biochem. Physiol.*, **12**: 107-115.
- JOSEPHSON, R. K., 1967. Conduction and contraction in the column of hydra. *J. Exp. Biol.*, **47**: 179-190.
- JOSEPHSON, R. K., AND S. C. MARCH, 1966. The swimming performance of the sea-anemone *Boloceroideis*. *J. Exp. Biol.*, **44**: 493-506.
- JUNGE, D. AND G. P. MOORE, 1966. Inter spike-interval fluctuations in *Aplysia* pacemaker neurons. *Biophys. J.*, **6**: 411-434.
- KANDEL, E. R. AND W. A. SPENCER, 1961. Electrophysiology in hippocampal neurons. II. Afterpotentials and repetitive firing. *J. Neurophysiol.*, **24**: 243-259.
- KENNEDY, D. AND DE F. MELLON, 1964. Synaptic activation and receptive fields in crayfish interneurons. *Comp. Biochem. Physiol.*, **13**: 275-300.
- KENNEDY, D. AND K. TAKEDA, 1965. Reflex control of abdominal flexor muscles in the crayfish. II. The tonic system. *J. Exp. Biol.*, **43**: 229-246.
- LENHOFF, H. M., 1961. Activation of the feeding reflex in *Hydra littoralis*. Pages 203-232. in H. M. Lenhoff and F. W. Loomis, Eds., *The Biology of Hydra and Some Other Coelenterates*. University of Miami Press, Coral Gables, Florida.
- LENHOFF, H. M., 1968. Chemical perspectives on the feeding response, digestion and nutrition of selected coelenterates, Pages 158-221 in B. Scheer and M. Florkin, Eds., *Chemical Zoology, Vol. II*. Academic Press, New York.
- MAYNARD, D. M., 1955. Activity in a crustacean ganglion. II. Pattern and interaction in burst formation. *Biol. Bull.*, **109**: 420-436.
- MORIN, J. G., 1969. Mechanisms controlling behavior and bioluminescence in the colonial hydroid *Obelia*. *Ph.D. thesis, Harvard University*, Cambridge, Massachusetts, 156 pp.
- PARKER, G. H., 1896. The reactions of *Metridium* to food and other substances. *Bull. Mus. Comp. Zool.*, **29**: 107-119.
- PARKER, G. H., 1917. The movements of the tentacles in Actinians. *J. Exp. Zool.*, **22**: 95-110.
- PASSANO, L. M., AND C. G. McCULLOUGH, 1962. The light response and rhythmic potentials of *Hydra*. *Proc. Nat. Acad. Sci.*, **48**: 1376-1382.
- PASSANO, L. M., AND C. B. McCULLOUGH, 1963. Pacemaker hierarchies controlling the behavior of hydras. *Nature*, **199**: 1174-1175.
- PASSANO, L. M. AND C. B. McCULLOUGH, 1964. Coordinating systems and behavior in *Hydra*. I. Pacemaker system of the periodic contractions. *J. Exp. Biol.*, **41**: 643-664.
- PASSANO, L. M., AND C. B. McCULLOUGH, 1965. Coordinating systems and behaviour in *Hydra*. II. The rhythmic potential system. *J. Exp. Biol.*, **42**: 205-231.
- ROBSON, E. A., 1961a. Some observations on the swimming behavior of the anemone *Stomphia coccinea*. *J. Exp. Biol.*, **38**: 343-363.
- ROBSON, E. A., 1961b. The swimming response and its pacemaker system in the anemone *Stomphia coccinea*. *J. Exp. Biol.*, **38**: 685-694.
- ROSS, D. M., AND L. SUTTON, 1961. The response of the sea anemone *Calliactis parasitica* to shells of the hermit crab *Pagurus barnhardus*. *Proc. Roy. Soc. London Series B.*, **155**: 266-281.
- RUSHFORTH, N. B., 1965. Behavioral studies of the coelenterate *Hydra pirardi* Brien. *Anim. Behav.*, Supp. 1: 30-42.
- RUSHFORTH, N. B., 1967a. Chemical and physical factors affecting behavior in *Hydra*: interactions among factors affecting behavior in *Hydra*. Pages 369-390 in W. C. Corning and S. C. Ratner, Eds., *Chemistry of Learning*. Plenum Press, New York.
- RUSHFORTH, N. B., 1967b. Behavioral responses of isolated tentacles of *Hydra*. *Biol. Bull.*, **133**: 449.
- RUSHFORTH, N. B., AND F. HOEFMAN, 1966. Behavioral sequences in the feeding response of *Hydra littoralis*. *Biol. Bull.*, **131**: 403-404.

- RUSHFORTH, N. B., 1971. Behavioral and electrophysiological studies of *Hydra*: I. An analysis of contraction pulse patterns. *Biol. Bull.*, **140**: 255-273.
- STEIN, R. B., 1967. Some models of neuronal variability. *Biophys. J.*, **7**: 37-68.
- STRUMWASSER, F., 1965. The demonstration and manipulation of a circadian rhythm in a single neuron. Pages 444-462 in J. Aschoff, Ed., *Circadian Clocks*. North Holland Publishing Co., Amsterdam.
- STRUMWASSER, F., 1968. Membrane and intracellular mechanisms governing endogenous activity in neurons. Pages 329-341 in F. D. Carlson, Ed., *Physiological and Biochemical Aspects of Nervous Integration*. Prentice-Hall, Englewood Cliffs, New Jersey.
- ÜNKULL, J. V., 1909. *Umwelt und Innerwelt der Tiere*. J. V. Springer, Berlin, 259 pp.
- WILSON, D. M., 1964. Relative refractoriness and patterned discharge of locust flight motor neurons. *J. Exp. Biol.*, **41**: 191-205.
- WRIGHT, E. B., AND P. D. COLEMAN, 1954. Excitation and conduction in crustacean single motor axons. *J. Cell Comp. Physiol.*, **43**: 133-164.